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The Use of Different Concentrations of Oxalic Acid to Decrease the Infestation of *Varroa Destructor* during Autumn Season as One of Integrated Management Methods

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Abstract. Trait results is applying different concentrations of oxalic acid on *Varroa* mite population parasitoid on honey bee which was conducted in the apiary of the College of Agriculture & Forestry for the year 2020. The concentration treatment of 4.2% showed the lowest mean number of mites falling on the hive board which amounted to 27.42 mites compared with the highest mean of 49.10 mites in the control treatment. This was reflected in the mean number of mites expected inside the cell. It was recorded in the control treatment which amounted to 4910 mites As for the relative effectiveness of the different treatments, the highest mean was recorded in the concentration treatment 3.5% with a mean of 86.84 compared with the lowest mean of 58.85 which was recorded in the concentration treatment 4.2%. As for the treatment efficiency, the highest mean efficiency was recorded 15.91% in the treatment concentration 4.2% compared with the lowest mean of 8.27% which was recorded in the 3.5% concentration treatment. Correlation statistical analysis showed some significant and non-significant differences among some characters in the different treatments.

Keywords: *Varroa* , Oxalic Acid , Mite, relative effectiveness, fall mite number, relative efficiency, *Apis mellifera* , bee.

Introduction

Varroa mite is an ectoparasite on bees (*Apis mellifera*) that parasitizes both adult bees and brood (Benard et al. 2001). Recent research has shown that European honey bees are affected by environmental factors surrounding them. As these effects are considered more important than genetic factors and these environmental factors were reflected in an increase The percentage of honey bees infested with Varroa mites (Francis et al., 2013 and Buchler et al., 2014), and there is a change in bee behavior and a lack of intelligence due to Varroa mites infection (Yang and Cox-Foster, 2007). Rosenkranz et al. (2010) mentioned that *Varroa* mite is a parasite that was originally confined to the oriental honey bee *Apis cerana*, and then turned into a new host i.e. *Apis mellifera* during the first half of the last century. Honey, due to its development of resistance mechanisms against many pesticides (Le Conte et al., 2020), and Adjlane et al. (2016), tested three concentrations of 4.2%, 3.2% and 2.1% of oxalic acid, and the mean percentage of efficiency obtained was 81 % and 72.1% and 65%, respectively. However, raising the acid concentration to 15%; that is, by 100 grams of oxalic acid / liter of sugar solution, led to the weakening of honey bee colonies. Thus, the colony lost 25% of its bees, and Aboushaara et al. (2017) concluded the effectiveness of oxalic acid ranged from 70.12% to 93.4% when oxalic acid was used in solutions with concentrations of 0.5%, 2.9%, 3% and 3.2%, where the concentration of 3.2% of the acid gave the highest killing rate of 95.81%. however, the killing rate of the two concentrations was 4.2% and 2.1 (81% and 46%) respectively which was used by distillation, evaporation or immersion of cellulose strips in oxalic acid solution. The beekeeping industry continually experiences colony mortality with annual losses as high as 43%. A leading cause of this is the exotic, ectoparasitic mite and *Varroa destructor*. Integrated Pest Management (IPM) options are used to keep mite populations from reaching lethal levels. However, and due to resistance and/or the lack of suitable treatment options. Novel controls for reducing mites are warranted. Oxalic acid for controlling *Varroa destructor* has become a popular treatment regimen among commercial and backyard beekeepers. Applying vaporized oxalic acid inside a honey bee hive is a legal application method in the U.S.A (Berry et al.

2022) . Accordingly, the current study aims at reaching the following:

1- Finding an effective solution to the problem of honeybees infected with *Varroa* mites under Mosul environmental conditions.
2- Finding an integrated program to control *Varroa* mites using oxalic acid treatment.
3- Evaluating the effectiveness concentrations of oxalic acid in controlling *Varroa* mites.

Materials and Methods

Experiments were conducted at the site of the apiary of the College of Agriculture & Forestry at the University of Mosul for the period 1/9/2020 to 5/12/2020 to study the effect of different concentrations of oxalic acid and their effects on bee colonies under the conditions of the city of Mosul. All experiments were designed according to the Randomized Complete Block Design Al-(Zubaidy & Al-Falahy, 2016) and divided into four blocks. Each block has three replicates (colonies). The results were analyzed according to the SAS program. A concentration of 60% sugar syrup was prepared in order to use a percentage of which to dissolve the amounts of oxalic acid prescribed for each treatment. Then it was divided into four groups; each group is 200 ml of sugar solution. The first group is the control group, while the second group is the 3.0% concentration where 6 gm oxalic acid is added to 200 ml, while the third group is the 3.5% concentration where 7 gm was added to 200 ml, and the fourth group is to prepare the 4.2% concentration where 8.4 gm was added to 200 ml. The number of fallen mites was daily calculated using the IPM/PUS board divided into white and black squares (spaces) manufactured by IPM INC stiky monitoring board UK where the study was photographed. The IPM monitoring board is designed with a Lancastroth hive board area (Fig.1). The plate is placed on a wooden slide under the screen bottom board for each replicate, and the number of mites falling on the white squares (areas) was calculated. The result was multiplied by three to get the total number (Sammataro et al. 2000). The following equations were used to get the studied results and characteristics according to the method of Moretto & Mello, (2000).

1- The expected number of mites in the hive = natural fallen mean * 100.
2- Relative effectiveness = fallen after treatment – pretreatment natural fallen mean / fallen after treatment *100.
3-The treatment efficiency = the treatment dead mite / dead mite total; viz. the fallen mite for each treatment + the fallen mite after the treatment.

Fig 1. The IPM INC stiky monitoring board is designed with a Lancastroth board area on top of wooden slide under the screen bottom board.

Results and discussion

The results of the late Table #1 related to the numbers of mites falling on the base of the hive when reading on 13/9/2020 and before applying the oxalic acid treatment show the homogeneity of mites in the different experiment treatments, and that the highest mean was 8.90 mites recorded in the control treatment compared to the lowest mean of 3.63 mites recorded in the concentration treatment 3.5%. However, the statistical analysis did not show any significant differences between the different treatments, and after applying the treatment with oxalic acid. A clear increase in the numbers of mites falling on the base of the hive was noticed when reading on 16/9/2020. This showed that the highest mean of the fall on the base of the hive was recorded in the 4.2% concentration treatment of 69.30 mites, which did not differ significantly from the 3.5% concentration treatment with a mean of 62.97 mites, but it was significantly superior to the control in which the lowest mean was recorded which amounted to 23.43 mites according to Duncan's multinomial test at the 5% probability level noting the clear role of oxalic acid. In its effect on dream fall at a mean temperature and humidity of 32.15°C and 27.80% respectively, when reading the above mentioning, the results of the table are shown when reading on 30/9 /2020, the highest mite shedding occurred in the 3.0% concentration treatment, as it recorded a mean of 90.67. On the contrary, it did not significantly differ from both the control and 4.2% concentration treatments with their mean adult mite 71.67 and 62.0 respectively. Yet, they were all significantly superior to the 3.5% concentration treatment in which the lowest mean was

recorded. The amount of 27.67 mites, that this to say, the fluctuation in the numbers of fallen mites, especially in the control treatment may be due to the lack of brood space and the tendency of the brood to live on the bodies of workers and as a result of the use of olive leaves to smoke on the sects during the examination which led to a lot of mite dropping in the control treatment, and also to the process of moving hives The apiary moved to sunny places while the experiment hives remained fixed in its place, which, in turn, led to the entry of stray bees from other colonies to the control colonies to them; thus, the number of fallen mites increased in them beside the role of the wire mesh base and the adhesive board in interaction with the hygiene behavior that raises the number of mites. The results of the table continuously show the gradual decrease in the number of fallen mites in oxalic acid treatments until the end of the season on 12/2/2020 to record a mean decrease in precipitation in the concentration treatment reaching 4.2% with a mean of 2.67 mites which did not significantly differ from the control treatment in which the highest mean was recorded of 32.67 mites, while the means reached 6.0 and 15 mites in the concentration coefficients of 3.0% and 3.5%, respectively. Thus, the above results were recorded at a degree of a temperature of 11.36°C and a relative humidity of 70.65%.

As for the general mean of the treatments, all oxalic acid treatments were significantly superior to the control treatment in which the highest mean was recorded which amounted to 49.10 mites, significantly compared with the lowest mean of 27.42 mites recorded in the 4.2% concentration treatment which did not differ from the 3.0% and 3.5% concentration treatments with their mean adults 29.09 and 31.97 mite. The results are in agreement with what was mentioned by Toomemaa et al.(2010), viz. most of the mites fell off after the first treatment. During the fall, experimental colonies containing young brood were treated once or twice with 0.5% oxalic acid solution which was highly effective in reducing mite populations (92.94 ± 0.01% and 91.84 ± 0.02%, respectively) with no observed toxicity to bees.

Table 1. The effect of different concentration of oxalic acid on the mite fallen duringtheAutumn season /2020.

treatment / date	Fallen mite number in the hive: Control	Concentration 3.0%	Concentration 3.5 %	Concentration 4.2%	total	
9/13	8.90 j-m	5.83 lm	3.63 m	4.53 m	5.73	h
9/16	23.43 h-m	36.67 f-l	62.97 b-g	69.30 a-e	48.09	bc
9/23	26.33 h-m	36.67 f-l	52.67 c-h	72.00 a-d	46.92	cd
9/30	71.67 a-d	90.67 ab	27.67 h-m	62.00 b-g	63.00	ab
10/7	96.67 a	51.67 c-h	67.33 a-f	41.67 d-i	64.33	a
10/14	78.67 a-c	39.33 e-j	37.67 f-k	25.67 h-m	45.33	cd
10/21	66.67 a-f	26.00 h-m	27.67 h-m	22.00 h-m	35.58	c-e
10/28	71.67 a-d	23.67 h-m	26.00 h-m	13.33 i-m	33.67	c-f
11/4	52.00 c-h	24.67 h-m	30.33 h-m	19.00 i-m	31.50	d-f
11/11	51.33 c-h	19.00 i-m	26.33 h-m	13.00 i-m	27.42	e-g
11/18	37.00 f-l	10.33 i-m	23.33 h-m	6.33 k-m	19.25	f-h
11/25	21.33 h-m	7.67 k-m	15.00 i-m	5.00 m	12.25	gh
12/2	32.67 g-m	6.00 lm	15.00 i-m	2.67 m	14.08	gh
total	49.10 a	29.09 b	31.97 b	27.42 b		

Values with similar letters for each adjective do not differ significantly according to Duncan's polynomial test at 5% probability level.
The sign (-) means the letters between them

The above results were related to the mean number of mites expected in the hive as shown in Table #2, as the mite numbers were low when read on 13/9/2020 and before applying the treatment. The lowest mean recorded in the concentration treatment was 3.5% of 363 mites compared with the highest mean of 890, the mite, which was recorded in the control treatment. Both treatments did not significantly differ from the concentration treatments 3.0% and 4.2% with their mean adults 583 and 463 mites respectively according to Duncan's polynomial test at a probability level of 5% where the mean temperature was 31.75°C and a relative humidity 35.90%. Noteworthy, applying the treatment with oxalic acid with its different concentrations, the results of the reading 16/9/2020 showed that the highest means of the expected numbers of mites were in the two concentration treatments recording 3.5% and 4.2%, and the adults were 6297 and 6930 mites. These were significantly superior to the control treatment with its mean of 2343 mites which did not significantly differ. Regarding the 3.0% concentration treatment with a mean of 3667 mites and with the progression of the effect of the treatment with oxalic acid, a continuous decrease was noticed in the number of mites expected in the hive with the progress of the autumn season and the decrease in temperatures to record the lowest means in the laboratories. The concentration was 4.2% with a mean of 267 mites which did not significantly differ from the treatment of concentration 3.0% and 3.5% with a mean of 600 and 1,500 mites. Also, they did not significantly differ from the control treatment in which the highest mean number of mites was recorded which amounted to 3267 mites. The effective role of different oxalic acid concentrations reduced the number of dreams before entering the winter sects, compared with the control treatment whose sects did not receive any treatment.

Table 2. The effect of different concentrations of oxalic acid in the expected number of mites in the hive during the Autumn season / 2020.

treatment / date	The expected number of mites in the hive				
	Control	Concentration 3.0%	Concentration 3.5 %	Concentration 4.2%	total
9/13	890 j-m	583 lm	363 m	453 m	573 h
9/16	2343 h-m	3667 f-l	6297 b-g	6930 a-e	4809.2 bc
9/23	2633 h-m	3667 f-l	5267 c-h	7200 a-d	4692 cd
9/30	7167 a-d	9067 ab	2767 h-m	6200 b-g	6300 ab
10/7	9667 a	5167 c-h	6733 a-f	4167 d-i	6433 a
10/14	7867 a-c	3933 e-j	3767 f-k	2567 h-m	4533 cd
10/21	6667 a-f	2600 h-m	2767 h-m	2200 h-m	3558 c-e
10/28	7167 a-d	2367 h-m	2600 h-m	1333 i-m	3367 c-f
11/4	5200 c-h	2467 h-m	3033 h-m	1900 i-m	3150 d-f
11/11	5133 c-h	1900 i-m	2633 h-m	1300 i-m	2742 e-g
11/18	37.00 f-l	10.33 i-m	23.33 h-m	6.33 k-m	1925 f-h
11/25	2133 h-m	7.67 k-m	1500 i-m	500 m	1225 gh
12/2	3267 g-m	600 lm	1500 i-m	267 m	1408 gh
total	4910 a	3197 b	2909 b	2742 b	

Values with similar letters for each adjective do not differ significantly according to Duncan's polynomial test at 5% probability level.
The sign (-) means the letters between them.

As for the general mean of the expected number of mites inside the hive, the lowest mean was recorded in the 4.2% concentration treatment which amounted to 2742 mites but it did not significantly differ from the 3.0% and 53% of the concentration treatments with a mean of 3197 and 2909 mites while all oxalic acid treatments were significantly superior to the control

treatment of which all the highest mean of 4910 mites was recorded. The results were identical to what was mentioned by Charriére and Imdorf (2002) that the use of oxalic acid by drip method on brood-free colonies in Switzerland gave mean efficiency of 97% for a solution containing 45 g of oxalic acid, while the percentage reached 96% for a solution containing 30 g of oxalic acid.

The above results were related to the relative effectiveness of the various treatments applied to the experiment as shown in Table #3 which states that when read on 16/9/2020 and after applying the treatment with oxalic acid at its different concentrations, it was noticed that the highest mean relative effectiveness was recorded in the 3.5% concentration treatment which amounted to 94.27%, followed by the 4.2% concentration treatment. Yet, with a mean of 93.46%, they did not morally differ from each other. Then, the concentration treatment was 3.0% with a mean of 84.10% and dream numbers were decreased within the sect. The relative effectiveness was negatively reflected which indicated the effectiveness of the concentration used, as the results of the table show the gradual decrease in the relative effectiveness continuously with the progress of the autumn season and to record the lowest means starting from the reading of 11/11/2020 until the reading of 12/12/2020. The analysis showed that there were statistically significant differences between the various treatments above for the results of the interaction according to Duncan's polynomial test at the 5% probability level. The relative effectiveness was positive despite the decrease in the number of mites. To begin a new phase of efficient negative activity especially in the 4.2% concentration treatment, it recorded a mean of 69.66-6% which significantly outperformed all other treatments according to Duncan test by reducing the number of mites falling on the base of the hive to its lowest level. This is a general equilibrium level where it recorded a mean capacity of 2.67 mites when read on 12/2/2020 which is a very low number compared to other treatments.

Table 3. Relative effectiveness of different concentrations of oxalic acid during the Autumn season / 2020

treatment / date	Relative effectiveness				
	Control	Concentration 3.0%	Concentration 3.5 %	Concentration 4.2%	total
9/13	-	-	-	-	-
9/16	62.02 t	84.10 kl	94.24 a	93.46 ab	83.63 e
9/23	66.20 s	84.10 kl	93.11 ab	93.71 ab	84.28 c
9/30	87.67 e-h	93.57 ab	86.88 g-i	92.69 b	90.20 a
10/7	90.79 c	88.72 ef	94.33 a	89.13 de	90.74 a
10/14	88.69 ef	85.18 jk	90.36 cd	82.35 m	86.65 b
10/21	86.65 g-i	77.58 o	86.88 g-i	79.41 n	82.63 e
10/28	87.58 f-g	75.37 p	86.04 ij	66.01 s	78.75 g
11/4	82.89 lm	76.37 op	88.03 e-g	76.16 p	80.86 f
11/11	82.49 m	69.32 r	86.21 h-j	65.15 s	75.79 h
11/18	75.95 p	43.56 v	84.44 k	28.44 w	58.10
11/25	58.28 u	23.99 x	75.80 p	9.4 y	41.87 j
12/2	72.67 q	2.83 z	75.80 p	-69.66 a*	20.43 k
total	78.50 b	67.06 c	86.84 a	58.85 d	

Values with similar letters for each adjective do not differ significantly according to Duncan's polynomial test at 5% probability level.
The sign (-) means the letters between them.

As for the general mean of the treatment, the statistical analysis showed the moral difference between the different treatments to record the highest mean relative effectiveness in the concentration treatment 3.5% with its mean of 86.84 compared with the 4.2% concentration treatment recorded a mean of 58.85 due to the negative number recorded when read on

12/12/2020. This result agrees with Nanitte's (2014) which concludes that a concentrated sugar syrup (60%) added with 4.2% oxalic acid was trickled by the means of a syringe (5 ml per Dadant-Blatt comb occupied by the bees) onto the comb top bars of broodless colonies. The efficacy averaged 96.8% and a weaker oxalic acid concentration resulted in a significant lower acaricidal effect.

The results of Table #4 showed the treatment efficiency related to the mean number of dead and fallen mites on the hive base before and after treatment show the inverse proportion of the mean treatment efficiency when read on 13/9/2020 with the mean number of fallen mites, as the highest mean was recorded in the control treatment, which significantly outperformed all other treatments. And the amount of 19.74, while the lowest mean was recorded in the concentration treatment 4.2%, which amounted to 4.83%, and the results show when read on 16/9/2020 the mean decrease in efficiency after applying the treatment with oxalic acid as a result of the high mean numbers of mites falling on the base of the hive to record the lowest mean in the concentration treatment 4.2 % mounting to 2.52% followed by the concentration treatment 3.5% with a mean of 3.59, while the concentration 3.0% recorded a mean ability of 5.17 and all treatments insignificantly differed from each other. The highest mean was recorded in the control treatment which amounted to 12.8. An increase in efficiency when read on 12/2/2020 in the concentration treatment recorded 4.2% with a mean of 49.51% which is originally associated with the lowest mean number of dreams falling on the bottom. This treatment significantly outperformed all the means of interaction for different treatments during the season and when read above to score the lowest mean of 15.36% in the control treatment which in turn significantly differed from the two treatments of concentration 3.0% and concentration of 3.5% respectively with their means of 31.96% and 22.31% according to Duncan's test. The polynomial is at the 5% probability level. As for the general mean of the treatment, the 4.2% concentration treatment showed moral superiority with a mean of 15.91% over all the other transaction means, and the lowest efficiency means were recorded in the 3.5% concentration treatment which amounted to 8.27%.

Table 4. Treatment efficiency of different concentrations of oxalic acid during the Autumn season / 2020

treatment / date	Control	Relative effectiveness Concentration 3.0%	Concentration 3.5 %	Concentration 4.2%	total	
9/13	19.74	8.92	6.24	4.83	9.88	
	h	t	f*	k*		e
9/16	2.81	5.17	3.59	2.52	6.02	
	m	i*	r*	v*		i
9/23	6.51	2.98	5.17	3.54	4.55	
	d*	t*	i*	s*		j
9/30	3.79	2.66	4.38	5.07	3.98	
	p*	u*	m*	j*		l
10/7	3.64	4.17	3.96	5.30	4.24	
	q*	n*	o*	i*		k
10/14	4.39	5.80	6.36	7.48	6.01	
	m*	h*	e*	z		i
10/21	4.61	7.63	7.74	10.09	7.52	
	l*	y	x	s		h
10/28	5.16	7.85	7.38	11.03	7.86	
	i*	w	a*	p		g
11/4	6.18	8.68	7.33	11.14	8.33	
	g*	u	b*	o		f
11/11	7.23	13.38	8.37	18.4	11.86	
	c*	l	v	i		d
11/18	10.94	21.06	10.84	31.46	18.57	
	q	g	r	d		c
11/25	11.8	27.74	13.85	46.5	24.97	
	n	e	k	b		b
12/2	15.36	31.96	22.31	49.51	29.79	
	j	c	f	a		a
total	8.61	11.39	8.27	15.91		
	c	b	d	a		

Values with similar letters for each adjective do not differ significantly according to Duncan's polynomial test at 5% probability level.
The sign (-) means the letters between them.

Reducing the number of mites at the end of the autumn season is an important criterion to ensure that sects successfully cross the winter which is related to the vital activity of the sects, especially the brood area and bee density. This is due to the production of a healthy brood during autumn and to be free from the infection by mites all of which are the basis for the production of a new generation of young bee workers. Accordingly, these workers will possess physiological characteristics especially their longevity which enable them to be able to successfully cross the winter season. Besides, a high bee density is necessary for colonies in winter to carry out the process of thermal adjustment within the colonies during low temperatures in winter and to reduce the daily natural death; and thus, the colony emerges in early spring with a capable bee density. On the exercise of vital activity, it can be said that these results are in agreement with what was mentioned by Nanetti (1999) and Imdrof et al. (2003). When directly applying oxalic acid as a spray to bees 1:1 in addition to 35 g of oxalic acid / liter of sugar solution, the effectiveness of oxalic acid was reached 95% in the absence of brood, and this result coincides with the experiment of Girişgin & Aydin (2010) where a group of organic acids were used including oxalic acid which increased its effectiveness upto 93.7% to reduce Varroa mites on bee colonies

Conclusion and Recommendations

The current study finally recommend that a 4.2% concentration of oxalic acid is very effective against *Varroa* mite but at the same time is affecting and decreasing honeybee colonies population. Thus, the 3.5% concentration of oxalic is recommended to be used which does not affect the honeybee population and which moreover gives good results in controlling *Varroa* mite during the treatment.

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